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Distribution of soil organic matter fractions are altered with soil priming

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ABSTRACT

Soil organic matter (SOM) plays a central role in mediating soil productivity through its impacts on nutrient cycling and retention, aggregate stability and water retention. Thus, management techniques or technologies including novel soil amendments could benefit farmers through the accumulation of carbon (C) and other nutrients in SOM. However, these same inputs can also lead to accelerated mineralization of native SOM through the process known as priming. This unresolved paradox may be due to the limited understanding of how different SOM fractions respond to priming and in which direction. In this study, we examine the response of functionally distinct SOM fractions to priming when soils are amended with lactobionate, a low molecular weight sugar acid byproduct of cheese manufacturing. Liquid-based ^{13}C lactobionate was added to an agricultural silty loam soil to study its persistence, priming effects, and response of different SOM fractions to lactobionate over 84 days. Cumulative soil carbon dioxide (CO_2) was greater in lactobionate-amended soils versus control and by the end of the experiment, 53% of added lactobionate was mineralized. In total, positive priming of 40% of extant SOM was observed from 14 to 84 days. Lactobionate-induced changes to SOM fractions were determined at days 14, 28, 56 and 84 of the incubation to examine if and how priming altered the distribution of C between fast and slow-cycling SOC fractions. In response to lactobionate, the total C content of the water extractable organic matter (WEOM) fraction initially increased by 100% from the dissolved lactobionate we added, but then declined and at a faster rate than other SOM fractions. In addition, the total C of the light-fraction particulate organic matter (LF-POM) fraction also declined. At the same time, we observed total C increases in the slower-cycling sand-sized POM (H-POM) and mineral-associated organic (MAOM) C fractions, in response to lactobionate additions. We also saw a marginal increase in total soil C in the lactobionate-amended soils. Our findings therefore suggest that the application of lactobionate to soils may induce positive priming of the faster cycling LF-POM and WEOM fractions, but also concurrent gains in the H-POM and MAOM C fractions associated with long-term persistence and relative resiliency to disturbance with no net loss of total soil carbon. Thus, the application of low-molecular weight C-based materials such as lactobionate presents an avenue to building more persistent SOM through its impacts on the internal cycling and transformation of SOM fractions.

1. Introduction

As the global climate crisis intensifies, managing soils to accumulate soil organic matter (SOM) is gaining widespread interest and investment as one potential climate and soil health solution. Increasing root biomass, especially living roots in managed soils is one approach that is receiving attention due to recent evidence that low-molecular weight bioavailable root compounds are more effective at building the C that contributes to long-term persistent SOM (Sokol et al., 2019; Villarino et al., 2021). At the same time, root exudates have been shown to stimulate SOM decomposition in a process known as priming, where SOM mineralization rates increase in response to newly added C (Dijkstra et al., 2013; Bengtson et al., 2012; Wang et al., 2016; Kuzyakov, 2010). As alternatives to more traditional organic amendments such as composts and crop residues, novel C-based soil amendments that exhibit properties similar to root exudates in terms of their solubility and low molecular weight are thus being considered (Olayemi et al., 2020). Central to understanding the net SOM balance and ultimately the impact on long-term soil C storage of such amendments, we need clarity surrounding how priming may influence distinct SOM fractions differently (Villarino et al., 2021). Not all SOM is functionally the same– some fractions of SOM may be relatively more important for aggregation

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while other fractions may be more critical for supporting active soil biological community. Still, much of our current understanding of SOM priming is limited to impacts on total soil C net balances. Unfortunately, this limits our understanding of which fractions of SOM are susceptible to loss and which fractions might instead be transformed within the soil, potentially altering the functional role of SOM.

The delineation of SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions has been a useful framework to improve our understanding of SOM and management recommendations for increasing soil C storage (Lavallee et al., 2020). Carbon associated with POM is likely to accumulate rapidly following crop harvest and residue inputs and responds to management changes, with a mean residence time on annual to decadal scales (Balesdent et al., 1987; Cambardella and Elliott, 1992). This fraction consists of both the light fraction, non-occluded POM containing partly decomposing plant materials, and the heavy fraction− sand-sized POM containing more decomposed plant and microbial residues that retains C via physical protection from microbial access through occlusion in macroaggregates (Balesdent et al., 1987; Cambardella and Elliott, 1992; Haddix et al., 2020; Mosier et al., 2021). The MAOM fraction stabilizes C onto clay and silt minerals via strong organo-mineral bonds that provide greater protection from microbial access and decomposition compared to POM, contributing to its relatively longer turnover time (Kögel-Knabner et al., 2008). Due to its slow turnover (on decadal - century scales), the MAOM fraction is well suited to more efficient long-term C storage as compared to POM, though some evidence suggests that MAOM can be desorbed, released back into solution under changing environment conditions, e.g anoxia, or following labile root inputs (Balesdent, 1996; Keiluweit et al., 2015). Understanding SOM dynamics through the lens of these fractions is vital to predicting management influences on long-term C accumulation.

While C is distributed and stored across these fractions, it can also be lost via mineralization. When exogenous C amendments are added to soils, microbial activity is often stimulated via rapid microbial anabolism of the added material (De Nobili et al., 2001; Stenström et al., 2001). This subsequent increase in microbial biomass may result in two simultaneous, non-mutually exclusive consequence to SOM dynamics: 1) increased production of microbial compounds that are preferentially sorbed and retained in the MAOM fraction (Cotrufo et al., 2013) or 2) elevated soil microbial biomass that enhances mineralization of existing SOM through priming (Blagodatsky et al., 2010). While priming should decrease extant (or native) C stocks, the response may vary among SOM fractions. In some instances, net increases in soil C with priming have been observed (Blagodatskaya and Kuzyakov, 2011). It may be that these observed increases in soil C with priming occur due to the cascading and interacting effects of stimulated decomposition transforming the distribution of SOM from ephemeral to the more persistent fractions. Because free POM is relatively unprotected, it is reasonable to expect that elevated microbial biomass will accelerate POM depolymerization, causing a priming of POM. In this process, POM becomes biologically altered, increasing its potential to be occluded, and thus protected, in aggregates (Lehmann and Kleber, 2015). Additionally, priming-induced POM depolymerization should increase the production of soluble C or dissolved organic matter (DOM) that can also be directly sorbed into the MAOM fraction or can be rapidly assimilated by microbes which may enter the MAOM fraction following their biomass turnover (Chantigny, 2003; van Hees et al., 2005; Kaiser and Kalbitz, 2012). As such, each of these fractions (POM, DOM, and MAOM) likely interact and influence one another to affect the outcome of C storage as described by soil continuum model (Lehmann and Kleber, 2015). Within this context, is it then possible that the potential priming of unprotected SOM fractions results in the formation of more persistent SOM fractions like MAOM?

To resolve this question, we conducted a laboratory incubation to examine SOM dynamics and interactions of different SOM fractions in response to soil amended with lactobionate. Lactobionate is a low molecular weight sugar acid derived from whey that is separated during cheese production. Large quantities of lactobionate are produced as a byproduct each year (180 million tons in 2013 alone), but current uses for this material fall short of the amount available, leading to waste (Dairy Processing Handbook, 2014; Olayemi et al., 2020). Meanwhile, few options exist for increasing low molecular weight C-rich inputs to soils. In addition, few studies have examined the influence of low molecular weight organic amendments across different SOM fractions with respect to both C stabilization and priming. Our objective was to study the persistence and priming effects of lactobionate in soils by examining C changes within SOM fractions over an 84-day incubation experiment. We added isotopically enriched ^{13}C-lactobionate to soils from an agricultural field and used quantitative tracing to determine lactobionate contribution to soil CO_2 efflux and distinct SOM fractions. We hypothesized that due to the absence of strong C protection mechanisms, a lactobionate-induced priming effect will lead to C depletion in WEOM and free-light POM fractions as compared to MAOM. We also predicted that lactobionate-amended soils would contain greater levels of C in their MAOM and intra-aggregate POM fractions as compared to unamended soils due to the potential lactobionate-induced activation of microbial activity and physical-chemical protection of C in these fractions via mineral sorption and occlusion in aggregates respectively.

2. Materials and methods

We obtained field soils to a depth of 20 cm from the USDA-ARS Central Great Plains Research Station located in Akron, CO (40.15 °N, 103.15 °W, 4540 feet elevation). The climate is semiarid, with an average annual precipitation of 420 mm (usclimatedata.com, accessed 2020). The soil is classified as a silty loam mesic Aridic Argiustolls of the Weld series (Calderón et al., 2015) or Calcic Kastanozems (WRB, 2006) with an average total C of 1.0%, total nitrogen (N) of 0.1% and pH of 5.7 (1:5 soil:water slurry). The inorganic C content of these soils are very low (less than 1% of total C) and negligible (Halvorson et al., 1997). Soils used for this incubation experiment were collected from a wheat field during the active growing season under high crop residue retention with no tillage management since 1995. Soils were temporarily kept in ziplock bags on ice during collection and transport and then stored at field-moisture level in a 4 °C refrigerator upon arrival at Colorado State University.

2.1. Incubation setup

To study the priming and C-stabilization effects of lactobionate in soils, we set up an 84-day laboratory experiment using a full factorial design that consisted of ^{13}C lactobionate-amended and unamended soils sampled destructively at four time points and replicated 5 times for a total of 40 incubation units. Additional 15 incubation units with no destructive sampling were used to capture soil CO_2 respiration dynamics and priming effects. These additional units consisted of ^{13}C lactobionate-amended soils, natural abundance ^{12}C lactobionate-amended soils, and unamended soils (control) replicated 5 times and combined in a full factorial design.

For all incubation units, soils were first sieved using a 2 mm-mesh sieve to homogenize samples and to remove large (>2 mm) surface and belowground organic material. Thereafter, sieved soils were weighed into 55 specimen cups (66.3 g of dry soil per cup) and then placed in 1 L Mason jars and lids were fitted with Swagelok thread connectors (Swagelok, Denver, CO). The incubation units were then placed in a constant temperature room at 25 °C for 7 days to allow for stabilization of soil respiration. Uniformly labeled (1215‰) as well as natural abundance lactobionate was used in this study and supplied in liquid formulation by Leprino Foods Company (Denver, CO). This formulation had a 65% moisture and 35% solids (lactobionate) content. Lactobionate is a low molecular weight sugar acid (<900 Da) that consists of 36.6% C, 4.8% potassium (K), pH of 6.7, and has no other nutrients.

We set up the lactobionate-amended incubation units by adding either labeled and unlabeled liquid lactobionate each at a rate of 0.00536 g C g^{-1} dry soil (0.015 g g^{-1} dry soil), increasing initial soil C content by 0.54%. We chose this rate for two main reasons; to reflect potential field application rates of lactobionate and to ensure that the microbial community in our low C soils (1%) were not saturated with lactobionate C that can often cause a negative priming effect. This rate is also in line with other similar studies (Ohm et al., 2007; De Graaff et al., 2010). Unamended control units received deionized water of the same quantity as the lactobionate treatments. Lactobionate was added to incubation units using a pipette and the soils were not mixed after lactobionate addition. All incubation units were kept in a dark constant temperature room at 25 °C for 84 days. The incubation units were maintained at 60% water holding capacity for the duration of the incubation. To prevent CO_2 accumulation, the destructively sampled incubation units were unsealed every 3 days for 3 h to allow for dissipation of the accumulated gas in the overhead space. Samples were destructively harvested at days 14, 28, 56 and 84 from the start of the incubation for further analyses discussed below.

2.2. Soil respiration and $\delta^{13}C$–CO_2

To capture soil CO_2 efflux and ^{13}C–CO_2 signature from both amended and control incubation units, 15 incubation units amended with ^{13}C lactobionate, ^{12}C lactobionate and deionized water (control) were tightly sealed and connected to a Picarro G2131-I Cavity Ring Down Spectrometer (CRDS; Santa Clara, California, USA). Prior to its use, the CRDS was calibrated according to the manufacturer's instruction. The CRDS was used to collect CO_2 concentration and the $\delta^{13}C$–CO_2 signature from the incubation jars within 10 min of connection. Soil respiration was measured every day for the first 15 days of the incubation (including two measurements on days 3 and 5 due to the rapid accumulation of CO_2 exceeding the 3% threshold) and every 2–3 days afterwards until the termination of the experiment on the 84th day. To obtain the CO_2 concentration at day 0, we measured headspace CO_2 concentration of each jar immediately after placing the specimen cups in the Mason jars. After each CO_2 measurement, all 15 incubation units were flushed with reconstituted, moistened and decarbonated air.

2.3. Soil fractionation and C and nitrogen content

Prior to SOM fractionation, we attempted to measure microbial biomass in all incubation units by the chloroform fumigation extraction method but due to methodological issues, the data was considered unreliable and thus not included in our analysis. However, we estimated that initial microbial biomass of our soils range between 180 and 200 mg C kg^{-1} soil from a prior study (Kallenbach et al., 2019). To determine changes in SOM fractions for each treatment over time, we employed a SOM fractionation scheme adapted from Haddix et al. (2020). By using a combination of size and density fractionation, four SOM fractions were sequentially obtained that include: water-extractable organic matter (WEOM) as a proxy for DOM, free-light POM (LF-POM), heavy POM (H-POM) and MAOM. We chose to separate POM into the light and heavy fraction because of their potential differences in their degree of decomposition, C:N, and aggregate protection (Christensen, 2001; Soong and Cotrufo, 2015). The fractionation process was carried out on 5.5–6.0 g of air-dried soil from each incubation unit and then oven-drying these samples overnight at 60 °C.

WEOM was obtained by adding 35 ml of deionized water to the oven-dried soils that were then shaken for 15 min and centrifuged at 1069 gfc for 15 min with a subsequent decanting of the liquid supernatant as WEOM. The soil samples post-WEOM were re-suspended in 35 ml of sodium polytungstate (SPT) at a density of 1.85 g cm^{-3} and centrifuged at 1069 gfc for 30 min. The floating material (LF-POM) was then aspirated off and rinsed four times to remove any remaining SPT. Following LF-POM removal, the soil samples were dispersed by shaking for 18 h with glass beads and 0.5% sodium hexametaphosphate to break all aggregates (Haddix et al., 2020). This was followed by the rinsing of dispersed samples over a 53-μm sieve to separate the H-POM (>53 μm) from MAOM (<53 μm).

The WEOM extracts were freeze-dried and all other SOM fractions (LF-POM, HF-POM, MAOM) were dried at 60 °C prior to weighing and analysis of C, N, and $\delta^{13}C$ on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS model: Optima; Micromass, Manchester, UK). The total fractions mass recovery was within ±5% of the initial mass. We also estimated the net carbon balance (carbon left in the soil minus carbon lost to respiration) by measuring total carbon and nitrogen on bulk soil samples (unfractionated) by dry combustion method on a LECO True Spec CN Analyzer (Leco Corp., St. Joseph, MI, USA).

2.4. Statistical analyses

We determined the relative contribution of lactobionate-derived C to soil CO_2 efflux and SOM fractions (^{12}C) using the isotopic mixing model as shown in the equation below (Balesdent and Mariotti, 1996):

Equation (1):

$$f_{\text{lactobionate}} = \frac{(\delta t - \delta c)}{(\delta L - \delta c)}$$

where $f_{\text{lactobionate}}$ is the lactobionate-derived C contribution to SOM fraction and CO_2. The δ_t and δ_C are the $\delta^{13}C$ of the specific SOM fraction and CO_2 sample from the lactobionate (δ_t) and the control (δ_c) treatment, respectively. The δ_L is the $\delta^{13}C$ of the initial lactobionate used for the incubation experiment (1215‰).

The lactobionate-induced priming effect intensity was also computed as a percentage of the control cumulative soil CO_2 respiration by using the equation below modified from Zhang et al. (2017):

Equation (2):

$$\text{Priming intensity } (\%) = \left(\frac{F_{SOM} * Q_{treatment} - Q_{control}}{Q_{control}}\right) * 100$$

Where F_{SOM} (native SOM-derived C) $= 1 - f_{lactobionate}$ and Q is the cumulative CO_2 respired from treatment ($Q_{treatment}$) or control ($Q_{control}$) in μg C–CO_2 g^{-1} soil day^{-1}.

To examine the effect of lactobionate on the C, N and $\delta^{13}C$ of the total carbon as well as the WEOM, LF-POM, H-POM and MAOM fractions, we fitted general linear mixed effect model using both treatment and timepoints (time) as fixed effects followed by pairwise comparison of control versus treatment at each timepoint using the Dunnett's test under the *emmeans* package in R. The level of significance (p) was set at 0.05 for all analyses. The dataset was evaluated for outliers, normality and equal variance assumptions using the diagnostics function in R (Q-Q plots, Residuals vs fitted plots). All figures were created using the ggplot2 package in R (Wickham, 2009). All analyses were conducted in R version 3.5 (R CORE TEAM, 2016).

3. Results

3.1. Soil respiration

Soils amended with ^{13}C-labeled lactobionate had higher soil CO_2 cumulative respiration across the entire incubation period as compared with unamended soils ($p = 0.001$) (Fig. 1). The majority of total respired CO_2 in the lactobionate-amended soils was derived from the added lactobionate (Equation (1); Fig. 1; Fig. S1). Most of the lactobionate contributions to CO_2 occurred within the first 14 days and then continuously declined, shown by the decrease in the $\delta^{13}C$–CO_2 throughout the experiment. The flux pattern of CO_2 derived from native SOM (^{12}C) in the lactobionate-amended soils was similar to the control (unamended soils) for the first 14 days of the experiment but afterwards

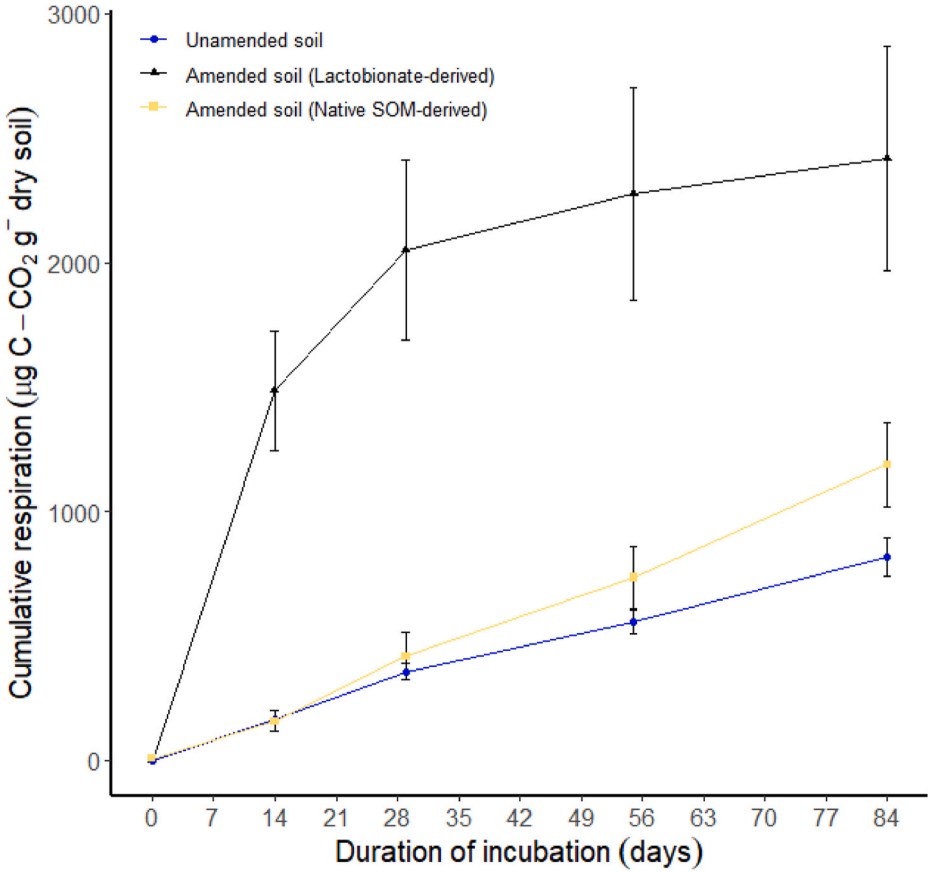

Fig. 1. Mean cumulative respiration of lactobionate-amended (treatment) and unamended soils (control) during an 84-day incubation (n = 5, error bars are standard error of the mean).

diverged from one another, with more CO_2 respired from the native SOM in lactobionate-amended soils as compared to unamended soils (Fig. 1).

3.2. Lactobionate persistence and priming intensity

At the end of the 84-day experiment, 52% of the added lactobionate added was respired as CO_2 in lactobionate-amended soils (Fig. S2), indicating that almost half of the lactobionate remained in the soil after 84 days. Similarly, 48% of added lactobionate was in the bulk ^{13}C soil after 84 days. Lactobionate-amended soils displayed a dynamic priming effect, shifting in direction and magnitude over the course of incubation. A negative priming effect (ranging from 0 to −40% relative to

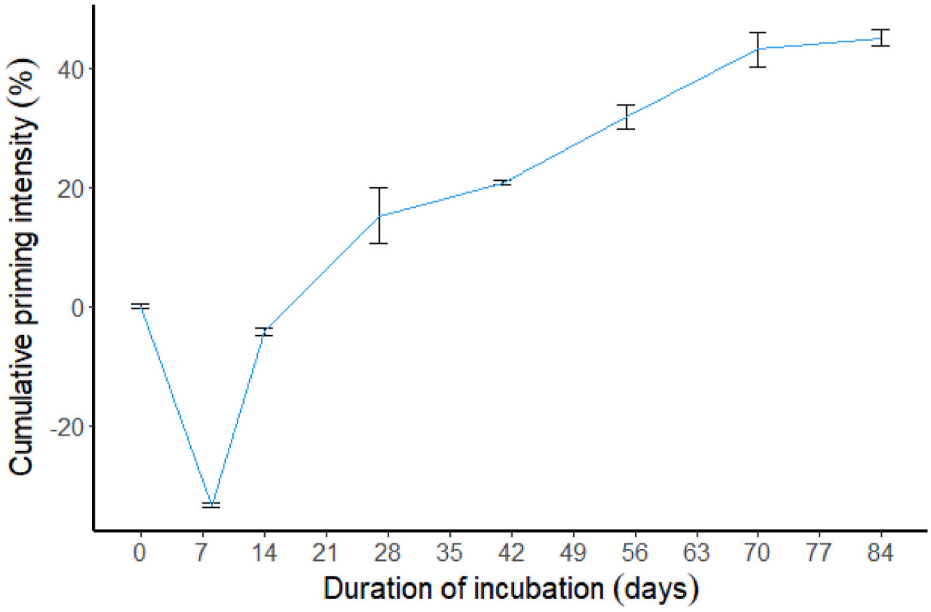

Fig. 2. Cumulative priming intensity of lactobionate-amended soils relative to unamended soils (n = 5, error bars are standard error of the mean).

unamended soils) was observed for the first 14 days of the incubation (Fig. 2). The magnitude of negative priming peaked roughly on the 9th day of the experiment. This was followed by a switch to positive priming (0–40%) in the lactobionate-amended soils for the remainder of the experiment. The intensity of positive priming increased steadily from day 14–67, and thereafter plateaued until the incubation was terminated on day 84 (Fig. 2).

3.3. Total SOM fractions and their responses to lactobionate addition

3.3.1. Heavy-particulate organic matter (H-POM)

Relative to unamended soils, lactobionate addition led to a maximum 40% increase in the total C content of the H-POM fraction as compared with unamended soils coupled (Fig. 3). The N content of the H-POM fraction was also higher in lactobionate-amended soils ($p = 0.004$) as compared with unamended soils and there was also a marginal interaction of treatment and time ($p = 0.06$) (Table 1). Further, the C:N of the total H-POM fraction for the lactobionate-amended soils declined over time, from 12.5 (day 14) to 8.19 (day 84) and this was lower than the unamended soils ($p < 0.01$) (Table 1). A steady decline in the $\delta^{13}C$ of the H-POM fraction was also seen across the incubation period (Table S2).

3.3.2. Light-fraction particulate organic matter (LF-POM)

Compared to the unamended soils, the lactobionate-amended soils decreased the total C content of the LF-POM fraction by a maximum of 25% (Fig. 3). Similar to C, N content of H-POM was lower in amended soils as compared to unamended soils and sampling time had no effect on both treatment and control soils with respect to LF-POM N content (Table 1, $p > 0.05$). The C:N of the LF-POM also differed by treatment ($p < 0.001$) and time ($p = 0.07$), with the amended soils having a higher C:N ranging from 17.7 to 16.1 as compared to 15 for control soils from days 14–84 of the experiment (Table 1). A steady decline in the $\delta^{13}C$ of the LF-POM fraction was also seen across the incubation period, starting at −8.21‰ on day 14 and declining to −15.29‰ on day 84 (Table S2).

3.3.3. Mineral-associated organic matter (MAOM)

The total C content in the MAOM fraction increased by a maximum of 15% under amended soils relative to unamended soils and this increase was consistent for the entire incubation period (Fig. 3). A similar time and treatment effect was observed for the MAOM N content of the amended soils ($p = 0.04$), which also had a lower C:N ($p < 0.01$) (Table 1). Like the POM fractions, lactobionate-amended MAOM fraction was enriched in $\delta^{13}C$ but declined steadily from 48.6‰ to 20.0‰ during the incubation period (Table S2).

3.3.4. Water-extractable organic matter (WEOM)

Lactobionate increased the total C content of the WEOM fraction to a maximum of 100% relative to control soils but an exponential decline was observed with time (Fig. 3). The opposite trend was observed for the N content as unamended soils retained more N content as compared to the lactobionate-amended soils with a significant time by treatment interaction ($p = 0.01$, Table 1). Similar to the other examined SOM fractions, $\delta^{13}C$ of the WEOM fraction declined steadily from 92.9‰ to −3.06‰ (Table S2).

3.3.5. Total soil carbon and nitrogen

There was a marginal significant increase in total carbon of lactobionate amended soils as compared to unamended soils ($p = 0.09$, Fig. 4). In contrast, there was no significant difference in soil nitrogen for lactobionate-amended and unamended soils (data not shown).

3.4. Effect of lactobionate on native (^{12}C) SOM fractions

Lactobionate additions changed the amount of SOM-derived C (^{12}C) across the SOM fractions, inducing increases, decreases or no change depending on the individual SOM fraction. The WEOM fraction had higher SOM-derived C in the lactobionate-amended soils as compared to unamended soils (Fig. 5). However, we saw a clear decline of WEOM SOM-derived C in the amended soils throughout the incubation period. SOM-derived C of LF-POM was significantly higher in unamended soils as compared to lactobionate-amended soils for the entire period of the incubation (Fig. 5). In contrast, both MAOM and H-POM fractions

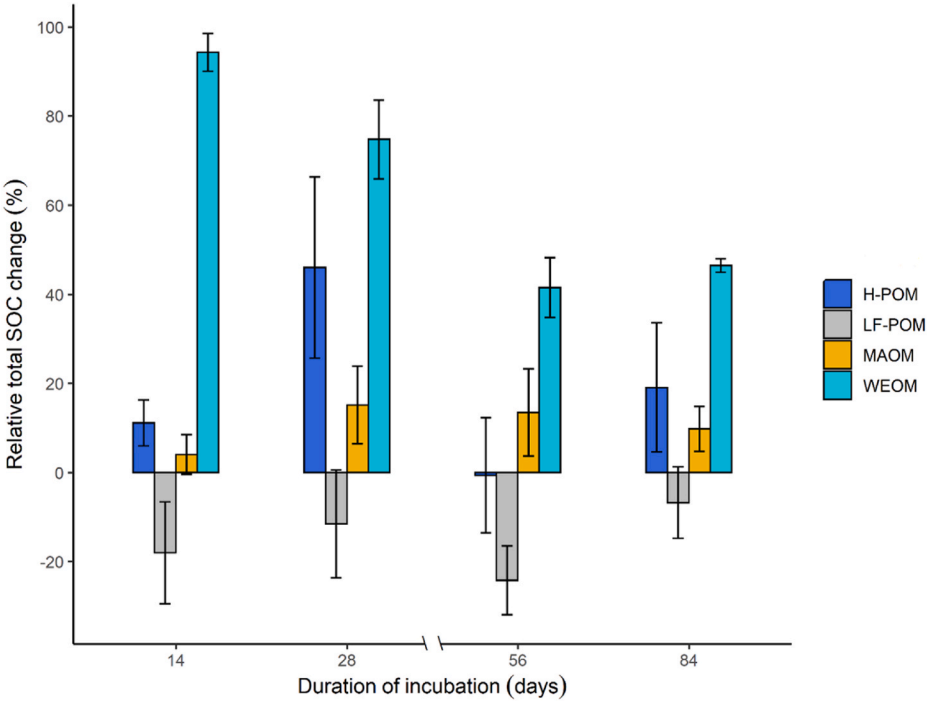

Fig. 3. Comparing changes in total (^{12}C and ^{13}C) mean soil organic carbon in lactobionate-amended and unamended soils for A: mineral-associated organic matter (MAOM); and B; water-extractable organic matter (WEOM) (n = 5, error bars are standard error of the mean).

Table 1
Effect of lactobionate on total N content and C:N ratios of heavy-fraction particulate organic matter (H-POM), light-fraction particulate organic matter (LF-POM), mineral-associated organic matter (MAOM) and water-extractable organic matter (WEOM). Data are means (n = 5) with standard error in parenthesis.

Treatment	Time (days)	H-POM N (mg N g^{-1})	H-POM C:N	LF-POM N (mg N g^{-1})	LF-POM C:N	MAOM N (mg N g^{-1})	MAOM C:N	WEOM N (mg N g^{-1})	WEOM C:N
Control	14	0.07 (0.00)	12.3 (0.37)	0.13 (0.01)	15.5 (0.19)	0.79 (0.01)	7.85 (0.08)	0.07 (0.01)	2.77 (0.43)
	28	0.06 (0.01)	14.1 (2.05)	0.14 (0.01)	16.0 (0.17)	0.70 (0.04)	8.07 (0.21)	0.07 (0.01)	2.90 (0.54)
	56	0.07 (0.00)	16.8 (1.16)	0.15 (0.02)	15.3 (0.31)	0.73 (0.02)	8.37 (0.08)	0.05 (0.01)	3.69 (0.86)
	84	0.07 (0.00)	13.5 (0.87)	0.13 (0.01)	15.2 (0.20)	0.70 (0.03)	8.17 (0.12)	0.10 (0.00)	1.44 (0.16)
Lactobionate	14	0.07 (0.01)	12.5 (0.42)	0.09 (0.01)	17.7 (0.28)	0.81 (0.04)	7.59 (0.06)	0.02 (0.00)	11.4 (0.39)
	28	0.09 (0.01)	12.6 (0.79)	0.11 (0.01)	17.1 (0.38)	0.86 (0.03)	7.41 (0.15)	0.02 (0.00)	11.3 (0.55)
	56	0.17 (0.05)	8.8 (2.05)	0.10 (0.01)	17.9 (0.82)	1.17 (0.15)	6.24 (0.84)	0.03 (0.00)	10.3 (1.07)
	84	0.26 (0.09)	8.2 (3.45)	0.12 (0.01)	16.1 (0.66)	0.90 (0.11)	7.32 (0.76)	0.06 (0.00)	5.82 (0.43)
		p-values							
Treatment		**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**
Time		**0.050**	0.508	0.665	0.079	**0.088**	0.666	**<0.001**	**<0.001**
Treatment × Time		0.067	0.091	0.378	0.162	**0.044**	0.152	**0.015**	**0.014**

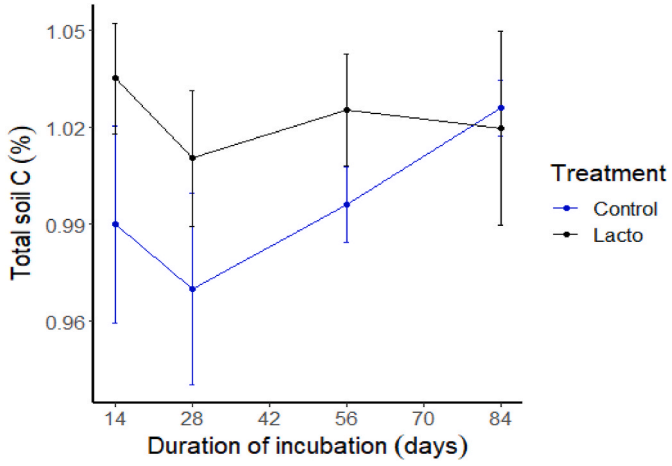

Fig. 4. Total soil carbon in lactobionate-amended and unamended soils (n = 5).

showed no significant differences in their SOM-derived C between lactobionate-amended and unamended soils but the lactobionate-amended soils trended higher for both fractions (Fig. 5).

3.5. Distribution of lactobionate-derived C in SOM fractions

The proportion of lactobionate-derived C contribution to SOM fractions in lactobionate-amended soils varied across fraction and by time (Fig. 6). The MAOM and WEOM fractions contained more lactobionate-derived C as compared to the POM fractions for the entire duration of the experiment. However, by the end of the incubation, the MAOM fraction contained the most lactobionate-derived C as compared to all other fractions. Less than 1% of lactobionate C was added to both the LF-POM and H-POM during the course of the experiment (Fig. 6).

4. Discussion

To effectively utilize soil amendments to increase SOM content, we need to better elucidate the mechanistic underpinnings of C dynamics through SOM fractions. Our study was thus designed to understand how labile inputs such as lactobionate affect the persistence or loss of soil C and its impact on different SOM fractions, given the different C protection mechanisms of these fractions.

4.1. Soil respiration and lactobionate persistence

As expected, cumulative soil respiration was greater under lactobionate-amended soils as compared to control soils, likely caused by the high bioavailability of lactobionate stimulating microbial activity and respiration (Blagodatsky et al., 2010; Daufresne and Loreau, 2001). This is supported by findings from a previous laboratory experiment where lactobionate-amended soils had on average 70 times more microbial biomass as compared to unamended soils over a 2-month period (Kallenbach et al., 2019). Despite elevated respiration with lactobionate, especially during the first two weeks of the incubation, nearly half of the labile lactobionate persisted in soil after 84 days. Other studies similarly show labile C materials such as glucose and cellulose persisting in soils (Kiem and Kögel-Knabner, 2003; Bore et al., 2019).

4.2. Lactobionate priming effects

We observed negative priming in lactobionate-amended soils for the first 14 days of the incubation experiment (Fig. 2). This may be attributed to microbial stimulation and resource utilization switch induced by the addition of lactobionate, a C-rich material that helped alleviate microbial C-limitation in a low C soil such as the one used in the experiment (Zhang et al., 2017). Initial negative priming after substrate addition has also been observed in other studies that have used similar quantities of labile C inputs (Cheng et al., 2014; Zhang et al., 2017). A switch from microbial lactobionate C utilization to native soil C respiration and subsequent positive priming began to occur around the 17th day of the experiment. It is worth noting that in our study, positive priming occurred even when there was still an abundant supply of lactobionate in the soil, including in the WEOM fraction. Thus, the switch from negative to positive priming may be less because of a change in substrate supply and more due to a shift in microbial communities. For example, dynamic priming effects have been explained by slow SOM-feeding K-strategy microbes replacing fast-feeding r-strategy microbes (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008). It is also possible that the shift to positive priming in our experiment could be attributed to N-limitations induce by lactobionate. Lactobionate contains no N and after a relative short period, the microbes likely became N

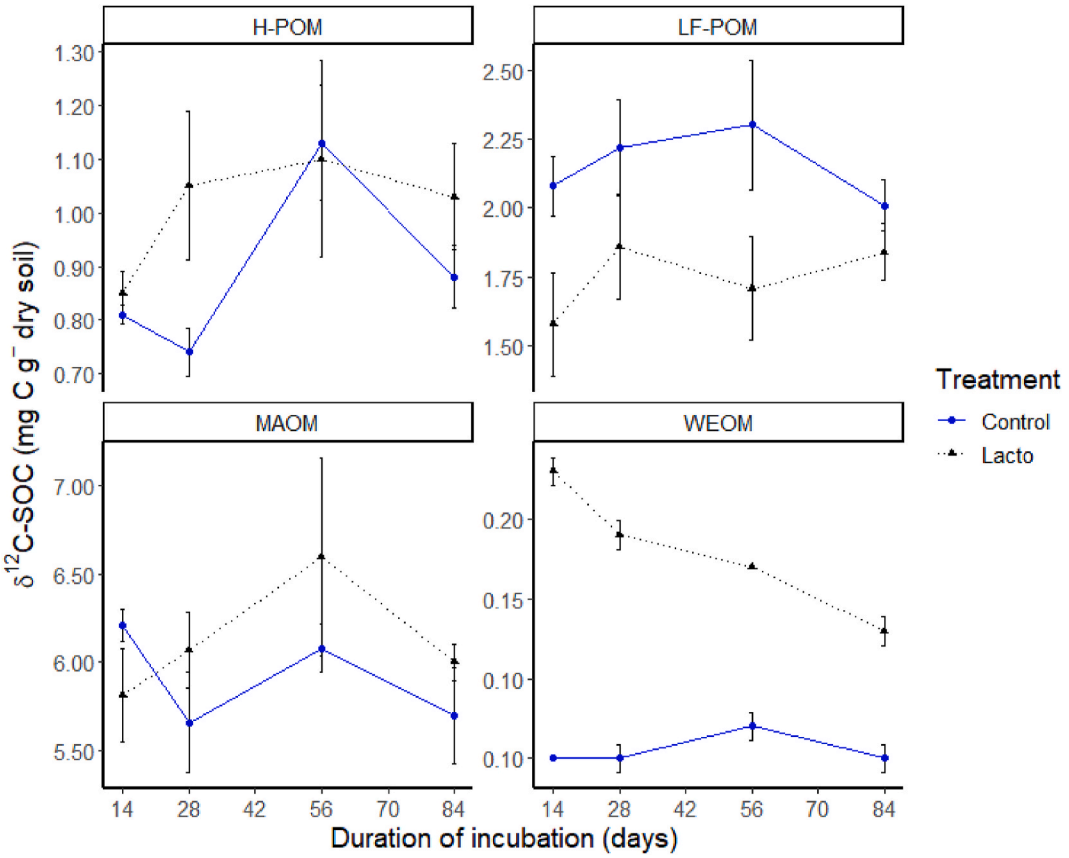

Fig. 5. Comparing changes in mean ^{12}C soil organic carbon (native SOC) in lactobionate-amended (Lacto) and unamended soils (Control) for heavy-fraction particulate organic matter (H-POM); light-fraction particulate organic matter (LF-POM); mineral-associated organic matter (MAOM); and water-extractable organic matter (WEOM) (n = 5, error bars are standard error of the mean).

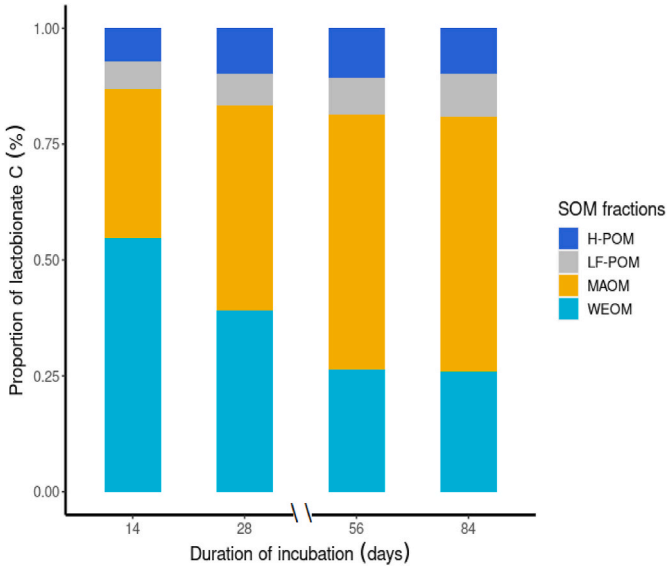

Fig. 6. Relative contribution of lactobionate derived-C to heavy-fraction particulate organic matter (H-POM); light-fraction particulate organic matter (LF-POM); mineral-associated organic matter (MAOM); and water-extractable organic matter (WEOM) fractions (n = 5).

limited and may mine native soil N to meet their nutritional requirements, as demonstrated in a number of studies (Craine et al., 2007; Guenet et al., 2010; Kuzyakov, 2010; Fontaine et al., 2011). However, we acknowledge that in actual field settings, lactobionate will mostly likely be applied in addition with mineral fertilization that may dampen the priming effects of lactobionate.

We hypothesized that the more unprotected WEOM and LF-POM SOM fractions would be the most susceptible to priming. While we cannot directly identify the source SOM fraction that is contributing to the CO_2 induced from positive priming, we can infer this by considering how the native SOM fractions change with priming (Fig. 4). The LF-POM fraction was the only native SOM fraction that decreased with the lactobionate amendment. This fraction has limited C protection compared to the other SOM fractions and should thus be more susceptible to priming with the lactobionate addition. The lactobionate-stimulated microbial community may have also been responding to N-limitation caused by high C inputs of lactobionate, potentially explaining the lower N content and higher C:N of LF-POM in lactobionate amended soil. While we did not observe an overall decrease in native WEOM-C in response to lactobionate and relative to the unamended soils, the native WEOM steeply declined over time in the amended soils, suggesting that native WEOM was also contributing to SOM-derived CO_2. Furthermore, neither the native MAOM nor the H-POM fraction decreased in response to lactobionate. Hence, our first hypothesis was partly supported as we saw a clear decrease in native LF-POM in response to priming and the native WEOM fraction appeared to respond to lactobionate-induced positive priming by the initial buildup but then its gradual depletion.

4.3. Lactobionate effect on SOM fractions

While the magnitude and direction of priming is crucial to understand C loss, fewer studies have comprehensively examined labile input-induced priming effects alongside C storage in specific SOM fractions. Examining the response of specific SOM fractions under priming

provides a mechanistic understanding of SOM dynamics as influenced by labile soil C amendments. We show that lactobionate decreases the amount of both total (^{13}C and ^{12}C) and native (^{12}C) LF-POM but generally caused an increase in the other SOM fractions, suggesting that lactobionate is accelerating the movement of newer, less protected C (LF-POM) into more protected fractions (H-POM and MAOM). We also show that lactobionate did not cause an overall decline in total soil carbon despite the priming effects observed on the LF-POM and WEOM fractions. Thus, while some C may be lost through priming, this appears to have stimulated native SOM transformations into more persistent fractions.

For instance, the relatively higher WEOM-C we observed with the lactobionate amendment (Fig. 5) may imply either an accelerated production or input rates to WEOM from stimulated POM decomposition, increased water-extractable microbial biomass, or from desorption of MAOM-C. Soluble labile materials such as lactobionate likely contributed directly to the initial increases we saw in total WEOM-C, however native WEOM-C was also higher with lactobionate amendments. Even though WEOM-C concentrations were consistently higher with lactobionate compared to unamended soil, both native- and lactobionate-derived WEOM-C decreased over time (Fig. 6). Labile WEOM compounds are rapidly lost via microbial CO_2 respiration (Kuzyakov et al., 2000; van Hees et al., 2005), but decreases in WEOM may also occur as WEOM moves out of solution directly into MAOM or indirectly via microbial WEOM utilization and then microbial biomass sorption (Cotrufo et al., 2015). While we cannot be certain if WEOM loss over time was due to respiration or sorption, given that the lactobionate-derived C in WEOM-C declined simultaneously with increase in MAOM-C (Fig. 5), it is clear that this fraction declines over time with labile input addition.

The changes we observed in POM with lactobionate further suggest that priming of available SOM fractions is simultaneously inducing the movement of unprotected C to more protected SOM fractions. Lactobionate amendment decreased the LF-POM fraction more so than any other fraction we measured, with observed decreases in the C and N content of both the total and the native LF-POM fraction relative to unamended soils (Fig. 5, Table 1). However, LF-POM can also contribute to H-POM and MAOM formation by the gradual depolymerization of this fraction via microbial activity. While it is unclear where LF-POM was lost to (H-POM, MAOM or CO_2), lactobionate-induced decreases in native LF-POM were matched by consistent increases in the H-POM with lactobionate addition (Table 1). The POM C:N ratio is often used as a proxy for the degree of plant residue decomposition, decreasing as decomposition advances, where compared to LF-POM, H-POM tends to contain more decomposed plant residues and microbial decomposition products (Golchin et al., 2018). The decrease in the C:N of the H-POM by lactobionate addition may therefore suggest that its decomposition is higher with lactobionate, potentially increasing its occlusion between and within aggregates and thus protection from further microbial attack. The decreased H-POM C:N could also be attributed to higher and more rapid turnover of microbial biomass induced from lactobionate. Although studies have suggested that the majority of microbial necromass and byproducts following microbial turnover in soil ends up in MAOM fraction, a new study has shown that the H-POM fraction can also retain a significant portion of microbial residues (Angst et al., 2019). Lastly, another explanation for the greater H-POM C levels with lactobionate addition could be a result of biogenic aggregation via microbial polymeric exudation that leads to more POM being occluded in aggregates (Deng et al., 2015; Cosentino et al., 2006; Kallenbach et al., 2019) protecting POM from microbial access. But if this were the case, we might not expect the large C:N decreases we observed over time with the lactobionate amendments. Regardless of the mechanism, our observations that lactobionate increases H-POM C suggests that our amendment is shifting the distribution of native C into the more persistent H-POM fraction, since no new POM can be created in our systems.

Similar to trends in the H-POM fraction, lactobionate-amended soils resulted in more native MAOM-C and N compared to unamended soils (Fig. 3). As compared to other fractions, the MAOM fraction also retained more lactobionate-derived C at the end of the experiment. The increase we observed in this fraction could be attributed to changes in three main sources of inputs to MAOM. First, the dominant constituents of MAOM are turnover residues and byproducts from microbial biomass and highly decomposed organic matter, both of which are relatively enriched in N compared to fresh and less decomposed organic matter. The lower MAOM C:N we observed in amended soils and relative to our POM fractions may thus be partly explained by the likely lactobionate-induced stimulation and turnover of microbial biomass that preferentially accumulate in MAOM. This explanation is supported by recent frameworks including the 'in vivo modification' pathway (sensu Liang et al., 2017) and the Microbial Efficiency-Matrix Stabilization (MEMS) model (Cotrufo et al., 2013) that have described MAOM formation as SOM passing through a microbial loop that increases its MAOM sorption potential. Similar to lactobionate, other low molecular weight compounds including glucose and root exudates have been shown to stabilize in soil through the mechanism described above (Bore et al., 2019; Sokol et al., 2019; Villarino et al., 2021). Secondly, the elevated lactobionate-derived MAOM-C may have also accumulated via direct sorption of non-microbial WEOM to mineral surfaces (Cotrufo et al., 2015; Haddix et al., 2020). However, ~25% of lactobionate was in the WEOM fraction after 84 d, suggesting that not all WEOM is directly sorbed, or at least is only temporarily sorbed, or that the ^{13}C-labeled WEOM fraction is being replenished from microbial biomass turnover.

Third, native MAOM-C increases could arise from enhanced POM decomposition. Our results support the idea that following labile C additions, enhanced decomposition or priming of LF-POM increases the feedstock of WEOM (whether derived directly from depolymerized POM or from increased microbial biomass from POM monomers) that could directly contribute to MAOM. The specific sources of C and N to explain the higher MAOM with lactobionate are likely a combination of all of the mechanisms described above. However, the decreases in WEOM and LF-POM and parallel increases in MAOM and H-POM contents and the shifts towards lower C:N ratio suggest that labile inputs induce transformations of existing C from easily accessible to more persistent C fractions. We contend that not only is the MAOM fraction not readily influenced by positive priming induced by lactobionate application but that it increases via a greater production of potential sources that contribute to MAOM. Given MAOM's strong organo-mineral bonding and the occlusion in microaggregates, funneling more C into MAOM in response to priming LF-POM could represent a potential unexplored pathway for enhancing SOM protection.

5. Conclusion

This study has described the effects of labile inputs on SOM dynamics by tracking the fate of ^{13}C lactobionate into soil CO_2 and SOM through its distinctive fractions (WEOM, LF-POM, H-POM and MAOM). While we observed a positive priming effect after 14 d, about 48% of the initial lactobionate remained in the soil after 84 d. Importantly, while lactobionate resulted in a net priming effect of the unprotected SOM fractions (WEOM and LF-POM), this priming effect had no effect on total soil C. However, this priming effect changed the fractions where C was stored, potentially increasing its long-term persistence as lactobionate led to more SOM in the more protected H-POM and MAOM fractions. In our study, we focused on a labile C addition under the rationale that this would more likely elevate microbial biomass growth and depolymerization rates with positive consequences to MAOM fraction. Our results suggest that C-rich soil amendments such as lactobionate may facilitate increased decomposition at the same time as building more persistent SOM.

Author contributions

OOP was primarily responsible for experimental design, data collection, analysis, and all manuscript drafts. CMK and MDW were responsible for experimental design, contributed to data collection and contributed to manuscript revisions. All authors contributed to manuscript draft revisions and contributed to conceptualization and design of experiment.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108494.

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